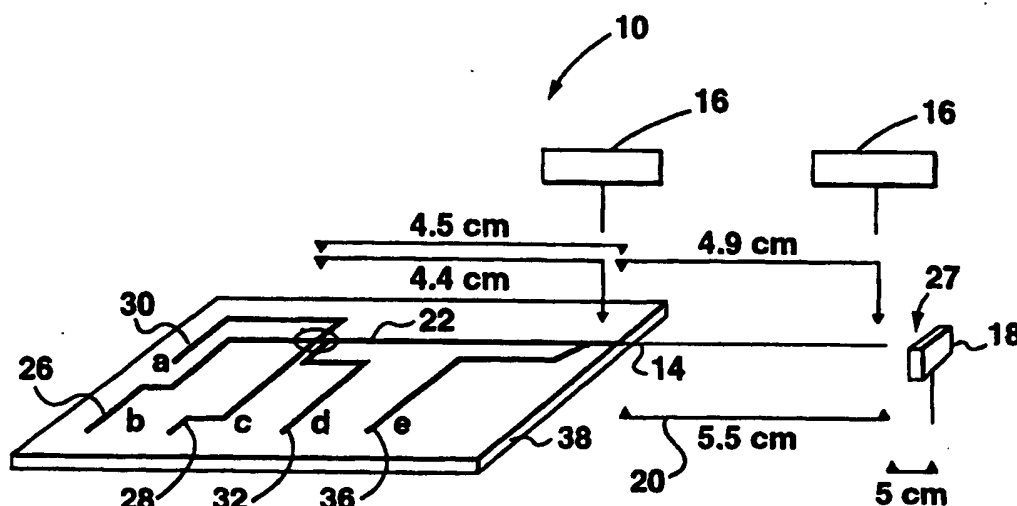




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(21) International Application Number: PCT/CA99/00868 (22) International Filing Date: 23 September 1999 (23.09.99) (30) Priority Data: 09/169,146 9 October 1998 (09.10.98) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 09/169,146 (CON) Filed on 9 October 1998 (09.10.98) (71) Applicants (for all designated States except US): UNIVERSITY OF ALBERTA [CA/CA]; Faculty of Science, Department of Chemistry, E3-44 Chemistry Building, Edmonton, Alberta T6G 2G2 (CA). INSTITUTE FOR BIOLOGICAL SCIENCES [CA/CA]; National Research Council, 100 Sussex Drive, Ottawa, Ontario K1A 0R6 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): TANG, Thompson [CA/CA]; #306-10713 - 80 Avenue, Edmonton, Alberta T6E 1V7 (CA). HARRISON, D., Jed [CA/CA]; W3-13 Chemistry, University of Alberta, Edmonton, Alberta T6E 1V7 (CA). BINGS, Nicolas [CA/US]; c/o Prof. Gary		Hieftje, Department of Chemistry, Bloomington, IN 47405 (US). WANG, Can [CA/CA]; 3A, 9111-112 Street, Hub Mall, Edmonton, Alberta T6G 2C5 (CA). OCVRK, Gregor [CA/CA]; 10539 - 85 Avenue #9, Edmonton, Alberta T6E 2K5 (CA). LI, Jianjun [CN/CA]; 101 Boul. Sacre Coeur, Hull, Quebec J8X 1C7 (CA). SKINNER, Cameron [CA/CA]; #204 Vanier Hse., Michener Pk., Edmonton, Alberta T6H 4N1 (CA). THIBAUT, Pierre [CA/CA]; 218 des Explorateurs, Aylmer, Quebec J9J 1M9 (CA). (74) Agent: BERESKIN & PARR; 40th floor, 40 King Street West, Toronto, Ontario M5H 3Y2 (CA). (81) Designated States: CA, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>Without international search report and to be republished upon receipt of that report.</i>	

(54) Title: MICROFLUIDIC DEVICES CONNECTED TO CAPILLARIES WITH MINIMAL DEAD VOLUME



(57) Abstract

A method is provided for joining a microchip device to a capillary tube. The microchip device has a capillary channel opening onto an edge surface of the device. A short hole is drilled into the edge surface, aligned with the capillary channel. The drilling is done with a flat bottom, preferably by a two-step drilling process. Then, the end of the capillary can be inserted into the hole so that its end is substantially flush with the flat bottom of the hole, thereby eliminating dead volume. Testing has shown that this connection provides very little band broadening of samples transported through the capillary channel into the capillary tube. The tip of the capillary tube can be tapered, so that it is suitable for use as an electrospray source for a mass spectrometer.

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**Title: MICROFLUIDIC DEVICES CONNECTED TO CAPILLARIES WITH
MINIMAL DEAD VOLUME**

FIELD OF THE INVENTION

This invention relates to microfluidic devices, and more
5 particularly, relates to an apparatus for and a method of coupling a
microfluidic device to an electrospray or other interface of a mass
spectrometer.

BACKGROUND OF THE INVENTION

10 Glass microfluidic devices have shown their vast potential
in the field of analytical chemistry in the last decade and enable a certain
amount of separation and analysis to be carried out. However, to augment
the analytical capabilities of microfluidic chip systems, it is necessary to
couple these devices to other instruments, and in particular it is desirable to
15 be able to couple them to mass spectrometers. Other uses for this type of
connection include, but are not limited to, coupling of the microfluidic
device to conventional Capillary Electrophoresis (CE) detectors, sample
introduction to a device, automation of a device and interconnections
between devices. In large part, these microfluidic devices find their greatest
20 utility in high performance separations. Therefore, connections to the
devices must have minimal dead volumes so that the efficiency of the
system is not compromised.

The coupling of separation methods with mass
spectrometry provides a powerful tool for rapid identification of target
25 analytes present at picogram levels in biological matrices, and structural
characterization of complex biomolecules ranging from small
pharmaceuticals to complex antibodies. Furthermore, mass spectrometry
using electrospray ionization (ESMS) has emerged as a sensitive technique
in a number of applications including the sequencing of peptides
30 comprising common or modified amino acids, and the analysis of short

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DNA oligomers. Further modification of ESMS has improved sensitivity substantially through the use of ionization techniques operating at sub-microliter flow rates, giving μ ESMS. The flow rates used and potentials applied in μ ESMS are compatible with CE, and this has led to development of CE- μ ESMS instruments, capable of initial separations followed by mass spectral analysis. A drawback of this approach is the 15-40 min. Separation times often required, which tends to underutilize the spectrometer.

Microchip technology has recently been applied to CE, generating an extremely powerful separation and sample pretreatment tool (chip-CE) with analysis time of a few seconds. Separations have been combined on-chip with sample dilution, derivatization, enzyme digestion, and a set of independent manifolds for separation have been integrated on to a single chip to give a form of multiplexed analysis. Thus, sample pretreatment can be automated within an integrated device, a feature which could offer significant advantages in sample preparation for mass spectrometry, particularly if the chip could be designed as an ion source within an ESMS system.

Mass spectrometry using electrospray ionization has emerged as a sensitive technique, providing peptide analysis in the low nanogram range for digested protein using sequence tags and data base searching (Mann, M., Wilm, M., *Anal. Chem.*, 66, 4390-4399 (1994)). Sequence information can be obtained from tandem mass spectrometric analysis where a given multiply-charged precursor ion is selected by the first mass analyzer and the fragment ions resulting from collisional activation with a neutral target gas (e.g. Argon) are transmitted into the second mass analyzer. The product ion spectra are characterized by easily identifiable series of fragment ions, and can be interpreted in the absence of protein or DNA sequence. Even in situations where only partial sequence is obtained, the sequence tag plus the peptide molecular weight can be used to locate the peptide in a given protein or data base. This combined approach was recently presented for the characterization of proteins from silver-stained polyacrylamide gels (Shevchenko, A., Wilm, M., Vorm, O., Mann, M., *Anal.*

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Chem., 68, 850-858 (1996)). Such advances have been facilitated by the introduction of micro-electrospray ionization operating in the low nL/min flow rate regime (Wilm. M. Mann, M., *Int. J. Mass Spectrom. Ion Proc.*, 136, 167-180 (1994)). Although this mode of sample introduction does not
5 require any prior analyte separation (e.g. Liquid chromatography or CE), the sensitivity of the micro-electrospray technique can be adversely affected by the presence of salts used in proteolytic digestion or by the simultaneous ionization of a large number of different peptides isolated from digestion or by the simultaneous ionization of a large number of different peptides
10 isolated from these digests. In addition, the mass spectra of unseparated digests are further complicated by the appearance of multiply-protonated molecules $(M+nH)^{n+}$ for each peptide, which significantly compromise interpretation if more than one peptide is initially present. The combination of a high resolution separation technique to micro-
15 electrospray sources thus confers a unique advantage in situations where both sensitivity and selectivity are desired.

The production of stable ionization conditions from micro-electrospray sources requires critical adjustment of low liquid flow rate (10-300 nL/min), column diameter, and field strength at the micro-electrospray
20 tip. Consequently, the coupling of separation techniques to micro-electrospray is best achieved using CE, which typically operates in a flow rate regime of less than 300 nL/min. Recent reports have demonstrated the applicability of the capillary electrophoresis-micro-electrospray mass spectrometry (CE- μ ESMS) approach for peptides and protein digests (Wahl,
25 J.H., Gale, D.C., Smith, R.D., *J. Chromatogr.*, 659, 217-222 (1994); Kriger, M.S., Cook, K.D., Ramsey, R.S., *Anal. Chem.*, 67, 385-389 (1995); Kelly, J.F., Ramaley, L.R., Thibault, P., *Anal. Chem.* 69, 51-60 (1997)). As a result of the high separation efficiencies obtainable with CE, analyses conducted using CE- μ ESMS typically yield 20-100 femtomoles mass detection limits in full-
30 mass scan acquisition mode and 100-200 femtomoles for tandem mass

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spectrometric analyses. This is a 10-fold enhancement of sensitivity compared to more conventional (i.e. non-micro) CE-ESMS interface, using a coaxial sheath design operating at flow rates of 2-10 $\mu\text{L}/\text{min}$.

The limited sample volume used in CZE (2% of capillary volume), results in concentration detection limits of approximately 1 μM , even at 20 femtomole mass detection limits. Improvement in sample loadings can be achieved using isotachophoretic preconcentration (Foret, F., Szoko, E., Karger, B.L., *J. Chromatogr.*, 608, 3 (1992); Foret, F., Sustacek, V., Bocek, P., *J. Microcol. Sep.*, 2, 127 (1990); Mazereeuw, M., Tjaden, U.R., Reinhoud, N.J., *J. Chromatogr. Sc.*, 33, 686 (1995)). This approach was successfully applied to the analysis of paralytic shellfish poisoning toxins present at low nM concentration levels in contaminated shellfish tissues, and enabled the injection of up to 1 μL on a single capillary arrangement (Locke, S.J., Thibault, P., *Anal. Chem.*, 66 6436 (1994)). On-line trace enrichment can also be obtained by loading large volumes of sample using microcolumns containing adsorptive media, followed by elution or electromigration onto a CE column. A review of different chromatographic preconcentrators has been presented recently (Tomlinson, A.J., Guzman, N.A., Naylor, S., *J. Cap. Elect.*, 6, 2247 (1995)). These methods provide satisfactory means to overcome many detection limit problems.

Capillary Electrophoresis is a well established method and provides a number of separation formats thus giving flexibility for the analysis of different biomolecules. It is well suited as a sample introduction device to a mass spectrometer (Banks, J.F., Recent Advances in Capillary Electrophoresis/Electrospray/Mass Spectrometry. *Electrophoresis*, 18, 1997; and Cai, J. and Henion, J. Capillary Electrophoresis - Mass Spectrometry. *J. Of Chromatography A.*, 703, 1995) At the most recent High Performance Capillary Electrophoresis Conference in Orlando Florida, several research groups reported on their efforts to directly interface microfluidic devices to mass spectrometers (Ramsey, R.S. And Ramsey, J.M. New Developments in Microchip ESI Mass Spectrometry; Figeys, D. And Aebersold, R. Microfabricated Devices Coupled to an Ion Trap Mass Spectrometer for the

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Identification of Proteins; and Liu, H., Foret, F., Zhang, B., Felten, C., Jedrzejewski, P. And Karger, B.L. Development of Microfluidic Devices for High Throughput ESI/MS). These groups have demonstrated that it is possible to obtain an electrospray directly from the microfluidic device, but
5 they did not demonstrate high efficiency separations. The inventors' experience with electrospray directly from the edge of a device, as in these other proposals, has shown that the droplet formed on the face of the device is sufficiently large that high efficiency separations are not possible due to the large mixing volume.

10 The effect of dead volumes is to distort the peak shape and increase band broadening. The maximum separation efficiency that can be observed with a microfluidic CE system joined to a capillary is limited by four principal sources of band broadening, namely longitudinal diffusion and effects of both injection and detection volume as well as any additional
15 dead volumes.

One proposal has demonstrated reasonable separations with a device that included a pneumatic nebulizer (Foret, F., Liu, H., Zhang, B. and Karger, B.L., Single and Multiple Channel Microdevices for Microanalysis by ESI/MS. HPCE, Orlando, Florida, February 1-5, 1998).

20 This still relies on forming an electrospray plume from the edge of the device, but combines this with a pneumatic or gaseous flow to improve nebulization of the emerging droplet, thereby reducing the droplet size and assisting in volatilization. A built in sprayer on the end of the chip is apparently simple and advantageous. However, it is believed that this can
25 never give the same performance as a tapered capillary tip. Such a capillary tip provides a smaller droplet size, thus less dead volume, and less band broadening. The length of the capillary can be changed to meet changing resolution needs since separation continues in the capillary. These combined devices would be able to exploit commercial micro electrospray
30 interfaces, with independent control over the electrospray operating

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parameters.

The literature has reported several methods used to join capillaries to microfluidic devices but to date they have shortcomings. Figeys *et al.* have constructed a butt joint to the edge of the chip with the use of a piece of Teflon tubing glued to the edge of the device as a guide sleeve and mooring point for the capillary. The capillary was used as an electro-osmotic pump for the introduction of protein digests to a MS device (Figeys, D., Ning, Y. And Aebersold, R., *Anal. Chem.*, 69, 1997, p. 3153-3160). This article gives information regarding the dead volume of the connection. It also acknowledged the presence of contamination that may have been due to the epoxy used to glue the capillary in position. This method also requires that the capillary and the channel be aligned to within a few microns and held in position by the glue, a difficult task at best. This type of connection has the additional shortcoming that it is not possible to directly examine the joint for the presence of debris, glue or dead volume. These problems render this joining technique impractical for most applications.

With silicon it is possible to form a connection with minimal dead volume. This was demonstrated by van der Moolen *et al* (van der Moolen, J.N., Poppe, H. And Smit, H.C., *Anal Chem.*, 69, 1997, P. 4220-4225). The SEM images of the interface presented in their article showed a tight connection with no apparent dead volume. The device was intended for correlation CE and no investigations were made for presence of band broadening introduced from the joint. Furthermore, the silicon device was used as an injector and performed the separation on the capillary. Unfortunately it is not possible to chemically etch deep structures into glass while retaining flat surfaces suitable for joining to a capillary so that the silicon procedure used by Moolen is inappropriate for glass. The problem with silicon devices is their inability to sustain the high electric fields that glass devices exploit for rapid separations. Consequently, a method to make low dead volume connections to glass devices is still needed.

SUMMARY OF THE INVENTION

Instrumental modifications are required to improve the analytical performance of the CE- μ ESMS interface in terms of ruggedness and speed of analysis. The present invention is based on the development of a compact and versatile, micromachined chip device to perform CE or other sample manipulation and then introduce the sample to a μ ESMS system, giving a chip-CE- μ ESMS hybrid system. The chips are thus an integral component of the electrospray ion source for the mass spectrometer, providing both sample treatment and ion source functions. The intent of the present invention is to develop a chip-ES interface which is easily manufactured, so that it can be made commercially at lower cost than current methods, and can increase utilization and sample throughput of rather powerful, but expensive instruments such as ESMS systems. This ES interface will be reusable, but readily replaced when required by the user.

Interfacing chips to μ ESMS would greatly expand the potential of both CE and ESMS for biotechnological applications requiring faster analysis time, enhanced sensitivity and selectivity. On-chip separations will provide for sample clean-up and separation of components to prevent interference in the mass spectrum, with a substantial reduction in analysis time (less than 5 and typically under 2 minutes). Minute sample and reagent consumption with less solvent and salt introduction at the interface should also lead to increased performance and efficiency.

To address this need, the inventors have developed a method of connecting fused silica capillaries to microfluidic devices. Silica capillaries were chosen because electrophoretic separations begun on the device can continue on the capillary and silica is transparent over a wide wavelength range. The initial invention was to develop an interface to MS that exploits the common micro electrospray. The results of MS experiments are presented.

In accordance with the present invention, a method is

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described for joining a capillary tube to microchip including at least one capillary channel that opens onto an edge surface of the microchip. The method comprises the following steps of:

(1) drilling a hole into the edge surface of the microchip, the
5 hole being aligned with the channel; and

(2) inserting an end of a capillary tube into the hole and bonding the capillary tube to the microchip.

Preferably, a flat-bottomed hole is formed in the microchip, and this is effected by: drilling a hole with a conically tipped drill bit, to form a hole
10 with a conical bottom, and removing the conical end face of the hole with a flat tipped drill bit having the same diameter as the first mentioned drill bit.

The method can include bonding the capillary tube to the microchip with an adhesive substance. Advantageously, the adhesive substance is applied from the exterior, by capillary action and is permitted
15 only to enter to the end of the capillary tube, without flowing substantially into an area between the end of the capillary tube and the capillary channel in the microchip.

Preferably, the method includes filling at least the end of the channel adjacent the edge surface of the microchip with an adhesive,
20 substance, to prevent substantial penetration of glass chips into the capillary channel. Conveniently, the substance is capable of being removed either by heating or by dissolution with a solvent.

It is preferred to provide the capillary with a tapered capillary tip, for use as an electrospray source for a mass spectrometer.

25 The hole can be drilled to a depth in the range of 2 to 5 times the diameter of the hole

Advantageously, the method includes mounting the microchip in a drill press, providing a drill bit in the drill press, lowering the drill bit until it is close to the edge surface of the microchip, and viewing
30 the drill bit through a magnifying means to ascertain that the drill bit is aligned with the capillary channel.

More preferably, the microchip device is mounted in a

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5 bracket, and the bracket is mounted on a Z axis translation stage, for movement in a horizontal X-Y plane, and the Z axis translation stage is adjusted to bring the capillary channel into alignment with the drill bit, for example by viewing the relative location of the drill bit to the capillary channel through a jeweller's loupe.

Another aspect of the present invention provides a combination device comprising:

10 (a) a microchip having at least one channel opening onto an edge surface thereon, and a hole extending from the edge surface into the microchip and aligned with the channel, the hole having a larger cross-section than the cross-section of the capillary channel; and

(b) a capillary tube mounted in the hole and bonded to the microchip.

15 Preferably, the hole is flat-bottomed and the capillary has a square end surface located substantially adjacent to the flat bottom of the hole.

The capillary is advantageously bonded in position by an adhesive substance, which does not extend to an area between the end surface of the capillary tube and the flat bottom of the hole.

20 In yet another aspect of the present invention, this device is provided in combination with a mass spectrometer.

It is to be appreciated that this invention is not limited to application with mass spectrometers. More generally, the method is applicable to any aspect of microfluidic technology in which it is desired to
25 connect a capillary tube to a microchip containing capillary channels. Such a device can be used to carry out a wide variety of different analytical and other techniques. The capillary tube itself need not necessarily have a free end, but could conceivably be connected at both ends to microfluidic chips, so as to provide an inter-connection between them. The capillary tube
30 enables a channel of any desired length to be provided, and can enable a

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variety of different processing to be carried out, e.g. by irradiation of the tube and/or detection of substances travelling through the tube.

A chip-ESMS interface also offers other advantages. Microfabrication allows for multiple sample treatment manifolds on a single chip, so that multiplexing of sample introduction into a single MS is feasible, thereby increasing throughput. Also, regular supply of mass calibration standards is possible via the chip, giving improved mass accuracy. Finally, a variety of more complex sample treatments, such as on-chip digestion of proteins or DNA can further automate sample preparation and introduction in the μ ESMS.

BRIEF DESCRIPTION OF THE DRAWING FIGURES

For a better understanding of the present invention and to show more clearly how it may be carried into effect, reference will now be made, by way of example, to the accompanying drawings which show a preferred embodiment of the present invention and in which:

Figure 1 is a schematic perspective view of a microfluidic device and a capillary tube in accordance the present invention, with Figure 1a showing a detail of the microfluidic device;

Figure 2 shows a perspective view of a drilling press including a bracket in accordance with the present invention;

Figures 3a, 3b and 3c show sequentially steps in drilling a hole in the edge of the microfluidic device;

Figure 4 is an image of a capillary inserted into a hole with a conical bottom, showing the dead volume;

Figure 5 shows electropherograms obtained using the combination device of Figure 4;

Figure 6 is an image of a capillary inserted into a hole with a flat bottom;

Figure 7 shows electropherograms obtained using the combination device of Figure 6;

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Figure 8a shows a schematic view of a microfluidic device-ESMS coupled directly with a micro-electrospray emitter;

Figure 8b shows a schematic view of a microfluidic device-ESMS interface using a coaxial sheath flow;

5 Figure 9a shows an ion electropherogram from the microfluidic device-ESMS interface shown in Figure 8a with extracted mass spectra for peaks highlighted in Figures 9b-9e;

10 Figure 10a shows an ion electropherogram from the microfluidic device-ESMS interface in Figure 8b with extracted mass spectra for peaks highlighted in Figures 10b-10j; and

Figure 11 shows schematically an apparatus for an alternative method of drilling a hole in the edge of a microfluidic device.

DESCRIPTION OF THE PREFERRED ENVIRONMENT

15 For the preferred embodiment of this invention, glass chips designed for interfacing directly to mass spectrometer were used, and these can have either a coaxial sheath flow arrangement or a sheathless interface (Figure 7). The chip can have a side channel at the end of the chip, forming a Y, that allows make up fluid flow and electrical control to assist the
20 electrospray function. The glass chips were prepared using the techniques and procedures previously discussed in the literature. The glass chips or microfluidic devices were designed to have the separation channel exit the edge of the chip to form an electrospray and as such are well suited to the present experiments. Figure 1 illustrates the overall layout of the device
25 and the experimental setup.

Figure 1 shows a combination device 10 comprising a microfluidic chip 12 and a capillary 14 connected thereto, in accordance with the present invention and as detailed below. Detectors 16 are indicated schematically, for detecting radiation from the chip 12 and the capillary 14.
30 A counter electrode 18 is provided for test purposes, but its use would be

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replaced by a mass spectrometer. As indicated, in this preferred embodiment, the capillary 14 has a length, indicated at 20, of 4 cm. The end of the capillary, as indicated at 22, is spaced at 5mm from the counter electrode 18.

5 The exact configuration of the microchip 12 is not critical to the present invention, and indeed any configuration of channels can be provided. Nonetheless, an exemplary channel configuration is detailed below.

10 The glass microchip 12 is formed from two layers. The channels are etched in one layer, and typically would have an approximately trapezoidal shape, or a cylindrical, oval or rectangular shape. A second glass sheet is then bonded to the top of the first sheet with the etched channels. The top sheet is usually formed with small holes, with a diameter of 1-2 mm, centered over the ends of the channels 26-36, so as to
15 provide connections to reservoirs. Reservoirs can either be formed by bonding directly onto the top glass sheet or by otherwise forming a connection to the small holes.

 A main microcapillary channel 22 extends from a junction with the capillary 14 indicated at 24. A buffer channel 26 is connected to the
20 other end of the main micro channel 22. A sample line is provided at 28, and on the opposite side of the channel 22 there is a sample waste 30. A further channel 32 is provided, but this was not used for experiments detailed below. A floating reservoir line 36 is connected to the main channel 22, adjacent the junction 24.

25 Junctions between the channels 28, 30 and 32 and the main channel 22 are shown in detail in Figure 1a. As shown, each of the channels 28, 30 and 32 forms a T-connection with the main channel 22, and they are all offset relative to one another. Between the channels 28 and 30, this creates a short section, indicated at 34 of the main channel 22. The
30 configuration of the sample supply line and reservoir 28 and the sample waste 30, enables a portion of the sample to be flowed through to 30, and, once the supply of sample is cut off, there will be left a plug or fixed length

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of the sample in the section 34.

Reference will now be made to Figures 2 and 3, to describe the detailed manner in which the capillary tube 14 is joined to the microfluidic device or chip 12.

5 The glass chip 12 was first prepared by filling one end of the main or separation channel 22 with dyed Crystal Bond 509 (Aremco Products - Crystal Bond is a trade mark of Aremco Products) to aid visualization and prevent plugging the channel 22. One to two drops of black ink from a Staedtler Lumocolor permanent pen were mixed with
10 approximately 1-2 ml of melted Crystal Bond to make the dyed Crystal Bond. Only the length of the separation channel 22 adjacent to the junction 24, where the device 12 was to be cut and drilled was filled, since the material was quite viscous. The device was placed on a hot plate (800 C) and by applying vacuum on the side or reservoir channel 36, the desired
15 segment of the main channel 22 was filled.

Crystal Bond was selected to fill the channel 22, over other possible options such as water and paraffin wax. It has a number of desirable attributes, namely: it is readily available; accepts dyes readily; it has a low melting point; forms free flowing chips reducing binding on the drill; and is
20 largely insoluble in water yet readily soluble in acetone.

Crystal Bond does have a disadvantage that prolonged exposure to water causes it to soften and expand. For this reason, the Crystal Bond is only allowed on the outside of the capillary and not to the area at the end of it, i.e. the dead volume 50. Other bonding materials can be used,
25 but Crystal Bond does have the advantage that it allows a joint to be disassembled by gentle heating.

With the channel 22 filled with Crystal Bond, the device 12 was cut at the appropriate position perpendicular to the separation channel 22 with a diamond glass saw. The newly cut edge surface 38 was sanded
30 smooth and flat with 220 grit and then 600 grit silicon carbide abrasive

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paper. This step facilitated locating the end of the separation channel and reduced the risk of the drill catching on the surface.

With reference to Figure 2, the device was then clamped vertically to a bracket 100 mounted on a horizontal Z axis translation stage 102 (Newport, Irvine, California) where the device is movable in a horizontal XY plane and the drill is movable vertically along the Z axis, with the main separation channel 22 held vertically and the end surface 38 on top.

As shown in Figure 2, the translation stage 102 is mounted on the base of a drill press indicated generally at 104. The drill press 104 is a conventional, high quality drill press. The bracket 100 is secured, as by screws to the top of the translation stage 102. The bracket 100 includes a main body 106 and a clamping plate 108, and a number of screws 110 are provided, to provide a simple clamping action. The chip 12 can then be clamped between the main body 106 and the clamping plate 108, with its edge surface 38 at the top, as indicated. Then, in known manner, the drill bit, indicated at 114, and mounted in the chuck of the drill press 104 can be brought down vertically to engage the surface 38.

In accordance with the present invention, a flat bottomed hole was cut, forming the junction 24. This was carried out using 200 μ tungsten carbide drills and flat tipped drills which were purchased from Tycom (Mississauga, Ontario) and had nominal tolerances of +0 -8 μ m. Alternatively flat tipped drills can be prepared manually by grinding the tip of the drill flat with a fine diamond wheel. A small jig was built to hold the drill bit and the wheel was rotated manually to avoid breaking the delicate drill. The manually flattened drill bits appear to produce better quality holes than the commercially prepared bits.

When drilling the hole, it was found necessary to use a high quality drill press 104 with no measurable runout. Such a press can produce holes with less potential for cracking of the wall of the hole and less drill bit breakage. The drill bit must follow the channel 22 within the chip 12; it was found that finer drills (200 micron) were better able to follow

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channel 22, having a nominal width of 45 microns, through the glass. It was also easier to center the drill bit. Due to their small diameter and brittle nature the glass powder must be removed from the drills.

The choice of capillaries compatible with the 200 micron
5 size of drill was limited to 185 μm OD and 50 μm ID. Unfortunately this inside area of the capillary does not provide a very good match to the cross sectional area of the separation channel. The channel has a cross sectional area of approximately 450 μm^2 whereas the capillary has an area of 1960 μm^2 or about 4.4 times larger. Such large mismatch in areas can lead to
10 unexpected band broadening due to inhomogeneous electric fields and distortions of the sample zone at the interface. The observed separation efficiency was about 95% of the predicted value for this volume mismatch, so the effect appears to be minor when the flat-bottomed connection is used.

Capillaries with a wider selection of ID are available in the
15 140 to 150 micron OD range but it was not possible to evaluate these capillaries. The 150 micron nominal OD drill bits that were tested in fact produced holes smaller than the available capillaries because of the relatively large negative tolerances on the drills. Larger drill bits (370 micron) produced holes suitable for (365 micron) capillaries which are
20 available with a wide range of ID. These capillaries appear to have a larger dead volume because of the larger diameter and hence larger area that is not sealed with Crystal Bond. The larger capillaries were not evaluated because interfacing the device to the mass spectrometer, the impetus for this research, is more convenient with the smaller diameter capillaries.

25 The Z axis translation stage 100 was used to center the channel on the end of the drill bit 114. A 20X jewellers loupe and side illumination were used, while the drill tip 114 was about 0.2 - 0.5 mm above the surface of the chip, to facilitate alignment. Once the drill 114 was accurately centered it was turned on (4000 RPM) and lowered until the tip

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touched the edge surface 38 (Figure 2), where it began removing glass. At this stage the drill 114 was raised and the face examined to ensure that the drill was on the center of the channel 22; if not, the chip 12 was removed, resanded and a new hole started.

5 If the hole was centered, a drop of water was placed on the surface of the device to help lubricate and cool the drill. The drill 114 was then lowered into the glass and allowed to bore approximately 1-2 drill diameters into the glass before it was raised. This process was repeated until a hole 40 of suitable depth (600 - 800 μm) was obtained (Figure 3a). The face
10 of the chip 12 was then cleaned with a paper towel to remove the glass powder produced during drilling. As detailed below both conventional conically tipped holes and flat bottomed holes were drilled for comparison purposes. For the flat bottomed holes, the conventional conically tipped drill bit 114 was then replaced with the flat tipped drill. A new drop of
15 water was placed on the device, the flat tipped drill was introduced and the bottom of the hole was flattened in one step (Figure 3b), as indicated at 42. Figure 3c illustrates that the bottom of the hole "fishtails" or widens out, if the flat tipped drill is forced to drill beyond the end of the hole left by the pointed or conical drill, as indicated at 66. The flat face of the drill bit is not
20 capable of removing the glass so the lower wall of the hole is enlarged which results in a poor connection and possibly increased dead volume.

The glass debris was removed from the hole by using one of the two following techniques. If there were no air bubbles in the hole then the device or chip 12 was inverted in a beaker of water and the glass
25 particles were allowed to settle out of the hole. This required a few hours. Alternatively a capillary (at least 25 μm smaller OD than the hole diameter) was used to flush the hole with filtered water (0.45 μm , Millipore). The chip was then placed on a hot plate and the Crystal Bond was melted and removed via the hole 40 at the end of the channel 22 with the aid of
30 vacuum. The device 12 was removed, allowed to cool and the residues of the Crystal Bond were washed clean with reagent grade acetone (Caledon Laboratories Ltd., Georgetown, Ontario).

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The end of the capillary 14 for connection at the junction 24 was prepared by sanding the end flat and square with 600 and 1200 grit silicon carbide paper, glass particles were flushed out with water. The capillary was glued into the device 12 by first placing the device 12 on a 10 x 10 cm scrap of glass to facilitate handling the hot assembly and then inserting the capillary tube 14 into the hole. The whole assembly was placed on a hot plate and allowed to heat to the Crystal Bond melting point (80 C). A small amount of the Crystal Bond was applied onto the surface 38 face of the joint and allowed to wick into the hole 40 until it nearly reached the end of the capillary 14. The rate of flow was controlled by adjusting the temperature. The assembly was removed and cooled with forced air to freeze the Crystal Bond.

Reference will now be made to Figures 4 and 6, which show the hole with a conical end, as drilled by a conventional drill and a flat-bottomed hole respectively. These holes in Figures 4 and 6 are indicated at 40a and 40b to distinguish them from one another and are also indicated as such in Figures 3a and 3b.

Referring first to Figure 4, the hole 40a has a conical end surface indicated at 41. The capillary 14 is shown inserted into the hole 40a. The end plane of the capillary is indicated at 46, i.e. the capillary 14 has a square end surface 46. The Crystal Bond securing the capillary 14 in position is indicated at 48. As shown, the conical end face 41 prevents the capillary 14 reaching the end of the hole. Consequently, there is a relatively large dead volume 50, having a frusto-conical shape between the end plane 46 of the capillary 14 and the capillary channel 22. This dead volume 50, as shown, is relatively large compared to the dimensions of the capillary channels, and is a significant multiple of the length along any capillary.

Referring to Figure 6, this shows a joint formed in accordance with the present invention. Here the hole 40b has a plane end face indicated at 43. Again, the capillary 14 is shown, secured in position

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with the Crystal Bond 48. It can now be seen that the end plane 46 with capillary 14 is very close to the bottom of the hole 43, so as to leave a relatively small dead volume 52. As explained below, this reduces band broadening due to dead volume effects.

5 The chip and capillary assembly was flushed with water, 0.1 M NaOH and then with the running the buffer for 30 min. for the complete assembly or apparatus. Partially decomposed (due to age) 0.1 μ M FITC labelled arginine diluted with buffer was used as a sample. Labelled arginine was prepared by mixing 7.63 mM arginine with 1.52 mM FITC (Sigma)
10 before allowing the mixture to stand overnight at room temperature and stored at 40C. 50 mM morpholine in deionized distilled water was used as a buffer with an unadjusted pH 9.5. Morpholine was chosen for this project because this buffer is suitable for MS detection of peptides.

 A sample was injected (from sample line 30 in Figure 1) by
15 applying -1.5 kV at the sample waste 28 with 26, 30 and 36 grounded and the counter electrode 18 was left floating. With sample present at 34, separation was performed by applying -7kV to the counter electrode 18, grounding the buffer reservoir 26, floating reservoir 36 and applying pushback voltages of approximately -550 V at 28 and 30, depending on liquid levels. During
20 separation an electrospray formed at the tip of the capillary 14. A flat piece of copper was connected as a counter electrode with an electrospray gap 27 of 5 mm, as shown.

 The channel 32, shown on the inset, is for a small volume injector, and was not used in this study and was left to float electrically.

25 Two devices, one where the hole was left with a conical bottom 40a from the pointed drill bit (Figure 4) and another one with a flat bottomed hole 40b (Figure 6) were used for the experiments and the experimental parameters are set out in Table 1. The difference in resolution caused by the different geometry of the two joints was evaluated by
30 comparing the resulting electropherograms. Two separate 2mW argon ion laser beams (448 nm) were focused to 40 μ m diameter spots on the capillary

- 19 -

14 and the device, respectively. Fluorescence signals were collected during separation, one on the separation channel 22, 4.4 cm from the injector 34, 1 mm before the function 24 and the second on the capillary 4.9 cm after the junction, 4mm from the tip of the 5.3 cm long capillary.

5 As detailed below in relation to Figures 8a and 8b, for electrospray operation an electrical connection needs to be made such that droplets emerging from the emitter are charged. This can be achieved by using the potential applied to the separation reservoir, 30 for example, to initiate both the separation and the ionization of analytes. As also detailed
10 below, an electrospray emitter can be prepared by tapering one end of the fused silica capillary to a smaller diameter. Alternatively, electrospray contact can be made using a gold-coating capillary emitter, butted to the chip which also offers independent control of the electrospray voltage.

The effect of dead volumes is to distort the peak shape and
15 increase band broadening for non-absorbing ions. The maximum separation efficiency within a microfluidic CE system is limited by longitudinal diffusion, the effects of both injection and detection volume as well as any additional dead volumes. A measure of the efficiency of a separation system is the ratio of the measured plate numbers to the maximum plate numbers
20 predicted by theory. The number of plates predicted by theory can be expressed as

$$N = \frac{L^2}{\sigma^2} \quad (1)$$

where L is the length of the capillary and σ^2 the variance of the peak, is given by,

$$25 \quad \sigma^2 = 2D_i t + I_{inj}^2 / 12 + l_{det}^2 / 12 + \sigma_{dv}^2 \quad (2)$$

where D_i is the diffusion coefficient of the analyte and t is analysis time, l_{inj} is the length of the injected sample and l_{det} is the detector spot size. The σ_{dv}^2

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term represents the effect of the joint's dead volume, and is of an unknown form that depends on the geometric shape of the dead volume and the electric field. Using equations 1 and 2 we calculated the expected number of plates using a separation column (injector to detector distance) length of
5 L=4.4 cm on the chip and 9.4 cm on the attached capillary. A D value of $3.9 \times 10^6 \text{ cm}^2/\text{s}$ was used for all of the components in the labelled arginine sample. The detector length was 40 μm and the injector length 34 was 400 μm .

The plate numbers were calculated from the well resolved
10 peaks in Figures 5 and 7, the percentage ratio of the measured to calculated peak efficiencies are presented in Table 2. From Figure 5 it is clear that the tapered bottom hole left by the pointed or conical drill bit (Figure 4) introduces significant band broadening. For example, the plate numbers for Peak 2 went from 40,000 to 15,500 on going from the device to the capillary.
15 As a result, this system only delivers about 12-17% of its theoretical efficiency. This should be contrasted with the data that was collected from the flat bottomed hole (Figure 6) as shown in Figure 7. In this case the plate numbers increased significantly from the device to the capillary and the observed efficiencies were in the range of 54-95% of the maximum. For
20 example, the number of theoretical plates went from 47,000 to 112,000 and from 71,000 to 117,000 for peaks 1 and 3 respectively. Theory predicts that the increased length of separation on going from the chip to the capillary should also give increased total number of plates. The results presented in Figure 7 agree with this and demonstrate that there is minimal dead
25 volume at the joint between device and capillary.

Given this data, it is clear that drilling into the device represents a useful method for connecting microfluidic devices to common CE capillaries. The requirements for minimized dead volume in the flow path can be easily met by using tipped drills to create flat bottomed holes.
30 This technique for connecting capillaries could be used for attaching standard capillaries for a wide range of applications. For example simple

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injectors could be attached to capillaries to give standard CE instrumentation the high efficiencies and rapid sample introduction, currently enjoyed only by complete microfluidic devices. Much of the current CE detector technology is based on UV absorption. With a capillary
5 attached to the device it is possible to exploit UV absorption as a means of detection. Currently this is a difficult task for microfluidic devices unless constructed from fused silica and employing multi-reflection absorption cells.

The coupling of sample separation with mass spectrometry
10 by means of electrospray ionization provides a powerful tool for rapid identification of analytes present in picogram levels in biological matrices, and structural characterization of complex biomolecules. Furthermore, ESMS has emerged as a sensitive technique in a number of applications including the sequencing of peptides comprising common or modified
15 amino acids, and the analysis of short DNA oligomers. Microfluidic devices, which could easily be connected to commercial electrospray nebulizers by common CE columns applying the here described coupling technique, would greatly expand the potential of both CE and ESMS for biotechnological applications requiring faster analysis time, enhanced
20 sensitivity and selectivity. The resulting on-chip separation and a wide variety of sample treatments, e.g. the on-chip digestion of proteins or DNA, would provide for sample clean-up and separation of components to prevent interference in the mass spectrum, with a substantial reduction in analysis time. Minute sample and reagent consumption, with less solvent
25 and salt introduction at the MS interface should also lead to increased performance and efficiency.

For electrospray modes, the diameter of the tip is critically important to the performance of the device. Hence, the ability to change capillaries, and tip diameters, is particularly useful, since the type of tip of
30 the capillary is quite fragile and susceptible to plugging and breakage.

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Referring now to Figure 8a, there is shown, schematically, a microfluidic device 60 to which is attached a capillary tube 62, as described above. The microfluidic device 60 is provided with three wells, indicated at A, B and C, connected by short channels to a main channel 64. A side channel 66 is connected to an additional buffer D.

Here, the capillary tube 62 had a length of 1-5 cm, and a 180 micron outside diameter (50 micron inside diameter). It was prepared by tapering one end of a fused silica capillary tube to a diameter of approximately 50 microns o.d. or less.

For electrospray operation, an electrical connection needs to be made such that droplets emerging from the emitter are charged. This can be achieved by using the potential applied to the separation reservoir to initiate both the separation and the ionization of analytes (Figure 8a).

A tapered tip can be provided by manually suspending a metal weight (15g) from one end of the capillary and melting the fused silica with the flame from a microwelding torch (see K.P. Bateman, R.L. White, and P. Thibault, Rapid Commun. Mass Spectrom., 11, 307-315 (1997)) to taper the free end of the capillary tube to an inner diameter of 15 μm (50 μm o.d.). The other end of the capillary 62 is then inserted in the chip 60 as described earlier.

In use, a solution to be tested was supplied at well A in Figure 7a. Typically, well B contains the separation buffer, and well C is the sample injection waste reservoir. A voltage applied between wells A and C creates a sample volume at the intersection of the channels. This plug is then driven towards the electrospray tip with a voltage applied between well B and the mass spectrometer. During the latter step a voltage can occasionally be applied to well D to assist the electrospray step. Typically voltages are in the range of 200 to 15,000 V on the chips, although 30,000 V has been demonstrated. Linear flow velocities of 0.01 to 15 mm/s can be achieved with these potentials. In order to improve the stability of the electrospray, an additional buffer solution was pumped from well D to

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increase the flow rate. The buffer solution was applied from the side channel (well D) with a flow rate of 50 nL/min. Also, a positive potential of the order of 5kV was applied to well B to effect for both separation and analyte ionization. The configuration of the chip design required that the flow from the side channel 66 be set to 150 nL/min or lower. Increase of flow rate above 150 nL/min resulted in improved signal stability, though the sensitivity was reduced as a result of peak broadening and counter flow during the electrophoresis separation. Alternatively, electrospray contact can be made using a gold-coated emitter butted to the chip which also offers independent control of the electrospray voltage (see K.P. Bateman, R.L. White, and P. Thibault, *Rapid Commun. Mass Spectrom.*, 11, 307-315 (1997)).

Reference will now be made to Figure 8b which shows an alternative arrangement. The microchip is indicated at 70 and the capillary tube at 72. As for Figure 8a, wells on the microchip are indicated A, B and C and are connected to a main channel 74. A side channel 76 is connected to a well D and an additional well E is provided. Here, the capillary tube, a fused silica transfer line 72, was generally longer and had a length of the order of 10-15 cm, with a 180 micron outside diameter and 50 micron inside diameter. It is connected to a T connector 78 including an inlet 80 for a sheath liquid. The sheath liquid flows in the inter-space between the CE column and the electrospray tip or needle at the tip of the connector 78 (see reference J.F. Kelly, S.J. Locke, L. Ramaley, P. Thibault, *J. Chromatogr. A*, 720, 409-427 (1996)). This approach provides an independent means of modifying the composition of the electrospray buffer for enhanced sensitivity, while simultaneously maintaining continuity of the voltage gradient across the CE capillary.

An ESI power supply 82 is connected between ground and the T connector 78. A CE power supply 84 is connected between ground and the well A as indicated from which to provide a potential for capillary

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electrophoresis.

As indicated schematically at 90, the electrospray could be directed to the inlet of a quadrupole mass spectrometer having a nitrogen curtain gas at the inlet, in known manner. The spectrometer could be, for example, a PE/Sciex API 300 (supplied by the Sciex Division of MDS Inc., of Concord, Ontario, Canada).

An example of separation conducted using the chip-ESMS interface shown in Figure 8a is presented in Figure 9. The total ion electropherogram for m/z 500-800 (Figure 9a) corresponds to the separation of a mixture of 9 peptides (injection of 64-180 fmol each). The main separation channel on the chip was 4 cm in length and neither the chip channels nor the electrospray emitter were coated. The peak width for individual components ranges from 12-20 sec. It is noteworthy that the separation efficiencies could be improved by increasing the length of the separation channel and reducing the tip of the electrospray emitter to smaller inner diameter. Extracted mass spectra taken for the peaks shown in Figure 9(b)-9(e) are also presented on the right panels.

The versatility of the chip-ESMS interface was also demonstrated for longer transfer line whereby this arrangement enables coupling to other types of mass spectrometric interfaces. An example of the chip-ESMS device using a 15 cm fused silica capillary coupled to a coaxial sheath flow interface is presented in Figure 10 for the separation of the peptide mixture shown in Figure 9. The coupling of the chip device via the co-axial sheath flow solvent delivery provides an independent means of optimizing the electrospray voltage or adding organic solvent to facilitate desolvation of the analyte. In this case both the chip of the capillary were coated with an amine reagent referred to as BCQ (see K.P. Bateman, R.L. White, and P. Thibault, *Rapid Commun. Mass Spectrom.*, 11, 307-315 (1997)) which not only prevents analyte absorption on the inner walls of the chip and capillary but enables the use of acidic separation buffers. Figure 10a shows the total ion electropherogram for the full scan analysis (m/z 400-900) of a mixture of 9 peptides each at 10 $\mu\text{g/mL}$. The ion electropherogram

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for the multiply-charged ions of each peptide are shown in Figures 10(b)-10(i). The mass spectrum for each peptide is dominated by singly and/or doubly protonated molecules of the corresponding peptides (peptides are designated in Figures 10(b)-10(j) using the single letter amino acid).

5 Reference is now made to Figure 11, which shows an alternative apparatus and method for drilling a hole in a microchip device. Here, the microchip device is indicated at 92, and is shown held in a clamp 94. Separate tubes 96 are connected to a pump (not shown) and are sealed by O rings 98 to the microchip device 92. A drill bit 99 is brought up against an
10 end of a channel 93 within the microchip 92, as indicated.

 Here, a steady stream of water or other fluid is supplied through the tubes 96 from the pump, connected to the various wells or inlets to the channel 93. This produces a continuous flow of fluid out from the free end of the channel 93, as indicated in Figure 11. Then, as the drill bit
15 98 is brought up against the channel, to drill a hole, the flow of fluid flushes away chippings, ground glass particles and the like.

 It was found that this technique prevented plugging of the exit channel, but did not always prevent the trapping of some particles within the channels on the chip, particularly the channels on the chip with
20 less than a 100 micron diameter and 20 micron depth. In general, it is expected that this technique would be better suited to larger size channels, where there is less likelihood of particles becoming trapped and a larger flow rate of flushing fluid can be maintained.

Table 1: Experimental parameters

Parameter	Value
Laser Power	2 mW per beam (nominal)
Ar ion at 488 nm	
Microscope Magnification	5X
Photo Multiplier Tube Filter	530 nm 10 nm bandpass
Separation Voltage	7 kV
Injection	1.5 kV for 1 s
Buffer	50 mM Morpholine
Sample	FITC Labeled dust and debris

Table 2: Average plate numbers and relative efficiencies of 6 selected peaks detected on the capillary (Figure 4 and Figure 6). Comparison of pointed and flat bottomed hole junctions between the device and the capillary.

Peak number	Pointed hole junction		Flat bottomed hole junction	
	Average plate number (n = 4)	Relative efficiency (%)	Average plate number (n = 4)	Relative efficiency (%)
1	18000	17	112000	84
2	15500	15	121000	95
3	15000	16	117000	94
4	12000	12	118000	69
5	9000	12	53000	54
6	9000	14	66000	76

CLAIMS:

1. A method of joining a capillary tube to microchip including at least one capillary channel that opens onto an edge surface of the microchip, the method comprising the steps of:
 - (1) drilling a hole into the edge surface of the microchip, the hole being aligned with the channel; and
 - (2) inserting an end of a capillary tube into the hole and bonding the capillary tube to the microchip.
2. A method as claimed in Claim 1, wherein step (1) comprises forming a flat-bottomed hole in the microchip.
3. A method as claimed in Claim 2, wherein step (1) comprises:
 - drilling a hole with a conically tipped drill bit, to form a hole with a conical bottom, and removing the conical end face of the hole with a flat tipped drill bit having the same diameter as the first mentioned drill bit.
4. A method as claimed in Claim 3, which includes bonding the capillary tube to the microchip with an adhesive substance.
5. A method claimed in Claim 4, wherein the adhesive substance is applied from the exterior, by capillary action and is permitted only to enter to the end of the capillary tube, without flowing substantially into an area between the end of the capillary tube and the capillary channel in the microchip.
6. A method as claimed in Claim 1, which includes filling at least the end of the channel adjacent the edge surface of the microchip with a substantially solid substance, to prevent substantial penetration of glass chips into the capillary channel.
7. A method as claimed in Claim 6, which comprises filling said end of the capillary channel with a substance capable of being removed by one of heating to melt the substance and being dissolved in a solvent.

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8. A method as claimed in Claim 1, which includes providing the capillary with a tapered capillary tip, for use as an electrospray source for a mass spectrometer.
9. A method as claimed in Claimed 1, which includes
5 providing a flow of flushing fluid through the channel, to at least reduce penetration of particles into the channel.
10. A method as claimed in Claim 3, wherein the method includes drilling the hole to a depth in the range of 0.9 to 10 times the diameter of the hole.
- 10 11. A method as claimed in Claim 10, wherein the method includes drilling the hole to a depth in the range of 2 to 5 times the diameter of the hole.
12. A method as claimed in Claim 10, which includes drilling the hole with a diameter in the range of 100 microns to one millimetre.
- 15 13. A method as claimed in Claim 12 which includes drilling the hole with a diameter which is one of 150 microns, 200 microns and 360 microns.
14. A method is claimed in Claim 1, which includes mounting the microchip device in a bracket, and mounting the bracket on a horizontal
20 Z axis translation stage, for movement in a plane perpendicular to the Z axis, and adjusting the translation stage to bring the capillary channel into alignment with the drill bit.
15. A method is claimed in Claim 14, which includes viewing the relative location of the drill bit to the capillary channel through a
25 jeweller's loupe.
16. A method is claimed in Claim 15, which includes, after determining alignment of the drill bit by visual inspection, engaging the drill bit with the edge surface to begin removing glass, raising the drill bit and examining the edge surface to confirm correct location of the drill bit.
- 30 17. A method is claimed in Claim 16, which includes, if the drill is incorrectly located, removing the microchip device, sanding the edge surface, mounting the microchip device again in the translation stage and

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repeating the steps of visually aligning the drill bit and checking to confirm correct location of the drill bit.

18. A method is claimed in Claim 16, which includes providing water as a lubricating fluid.

5 19. A method is claimed in Claim 16, which includes drilling the hole in a number of successive steps, each step comprising extending the depth of the hole in the range 1 to 2 times the diameter of the drill bit, and removing the drill bit out of the hole after each step, to ensure discharge of powder created by drilling.

10 20. A combination device comprising:

(a) a microchip having at least one channel opening onto an edge surface thereon, and a hole extending from the edge surface into the microchip and aligned with the channel, the hole having a larger cross-section than the cross-section of the capillary channel; and

15 (b) a capillary tube mounted in the hole and bonded to the microchip.

21. A combination device as claimed in Claim 20 which includes: (c) a side channel that can be used for at least one of: introducing at least one of a buffer and a solvent immediately upstream of
20 the capillary tube; and applying a voltage.

22. A device as claimed in Claim 20, wherein the hole is flat-bottomed and the capillary has a square end surface located substantially adjacent to the flat bottom of the hole.

23. A device as claimed in Claim 22, wherein the capillary tube
25 is secured by means of an adhesive substance between the capillary tube and the hole.

24. A device as claimed in Claim 23, wherein the adhesive substance does not extend to an area between the end surface of the capillary tube and the flat bottom of the hole.

30 25. A device as claimed in Claim 20, wherein the hole has a

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length which is in the range of 2 to 5 times the diameter of the hole.

26. A device as claimed in Claim 25, wherein the hole has a diameter of 200 microns.

27. A device as claimed in Claim 20, wherein the microchip
5 comprises two glass sheets bonded together, with a network of channels etched into one glass sheet including a main channel to which the capillary tube is connected with the other channels connected to the main channel.

28. A device as claimed in Claim 27, wherein the other glass sheet includes a plurality of openings, aligned with free ends of said other
10 channels, providing reservoir connections.

29. A device as claimed in Claim 20, in combination with a mass spectrometer

30. A device as claimed in Claim 29, which includes a power supply connected to the free end of at least one or said other channels, for
15 providing a potential, for both electrophoresis and for generating an electrospray from the capillary tube.

31. A device as claimed in Claim 29, which includes a power supply connected to the free end of at least one or said other channels, for providing a potential for electrophoresis, and a second power supply
20 connected to the free end of the capillary tube, for providing a potential for electrospray ionization.

32. A device is claimed in Claim 3, which includes a connection T at the free end of the capillary tube and an inlet to the connection T for a sheath buffer fluid.

25 33. A method of analyzing a sample, the method comprising:

(a) Providing a microchip having at least one channel opening onto an edge surface thereof and a hole extending from the edge surface into the microchip and aligned with the channel, the hole having a larger cross-section than the cross-section of the capillary channel, a
30 capillary tube mounted in the hole and bonded to the microchip, and a mass spectrometer;

(b) Supplying the sample to the channel in the microchip

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and applying a voltage across at least the channel, to drive the sample along the channel and out of the free end of the capillary tube to form a stream of ions;

- (c) Passing the ions into a mass spectrometer and analyzing
5 the ions to obtain a mass spectrum.

34. A method as claimed in Claim 33, which includes:

(1) Providing a supply of a sheath buffer fluid at a free end
of the capillary tube;

(2) Providing a voltage across the channel on the
10 microchip, to drive the sample along the channel; and

(3) Providing a separate potential at the free end of the
capillary tube, to form a stream of ions for analysis.

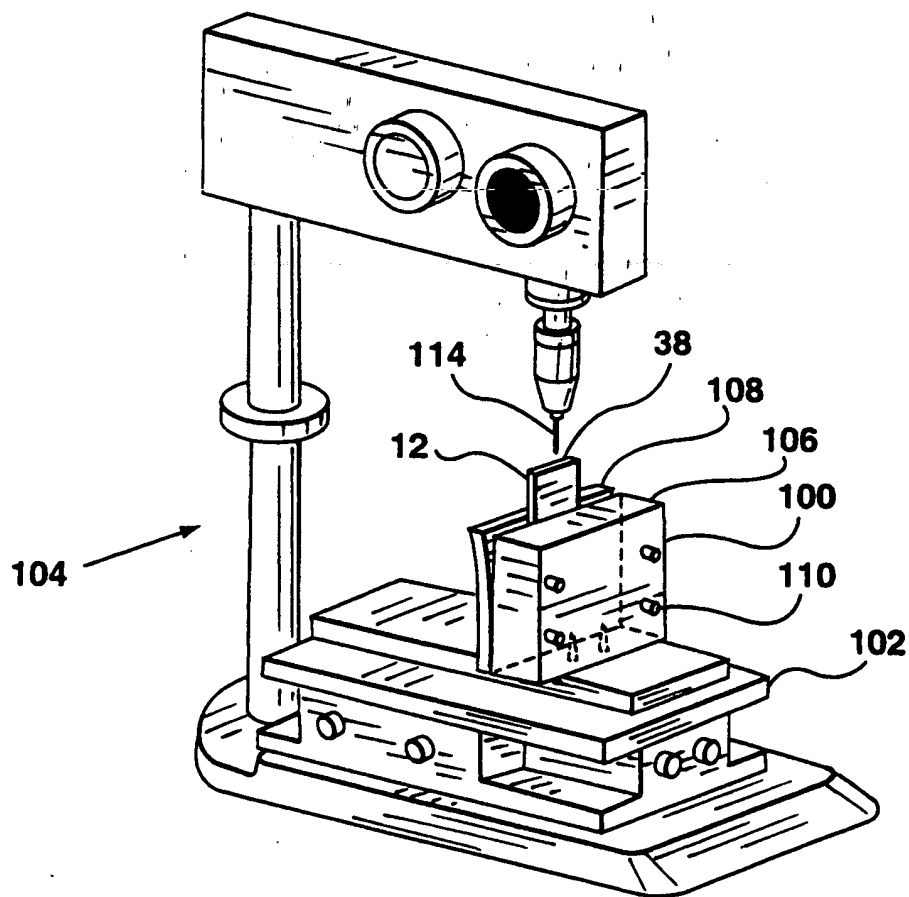
35. A method as claimed in Claim 33, which includes:

(1) Passing the sample along a main channel on the
15 microchip to the capillary tube;

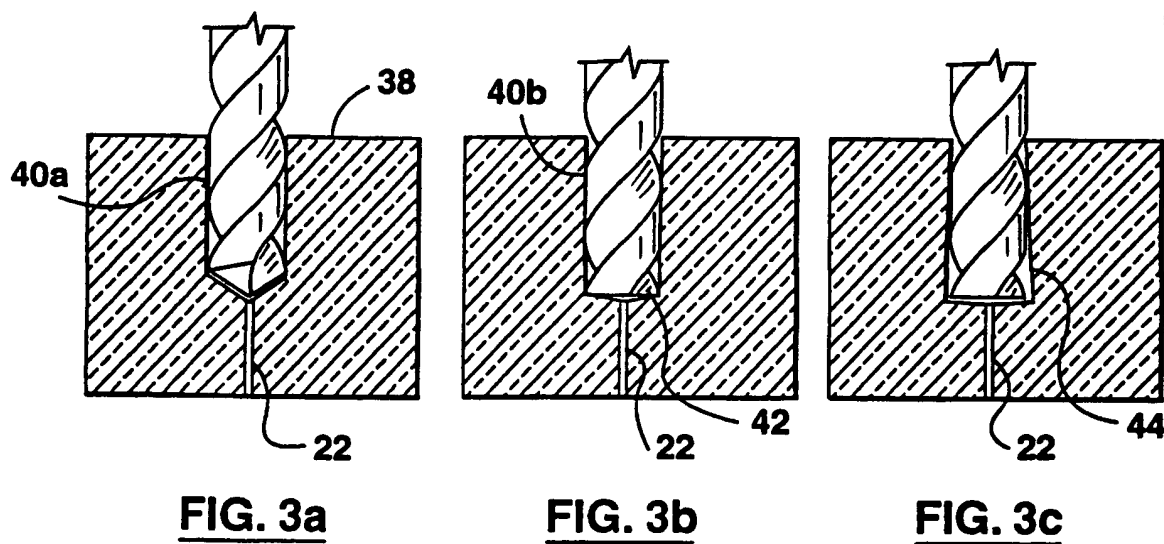
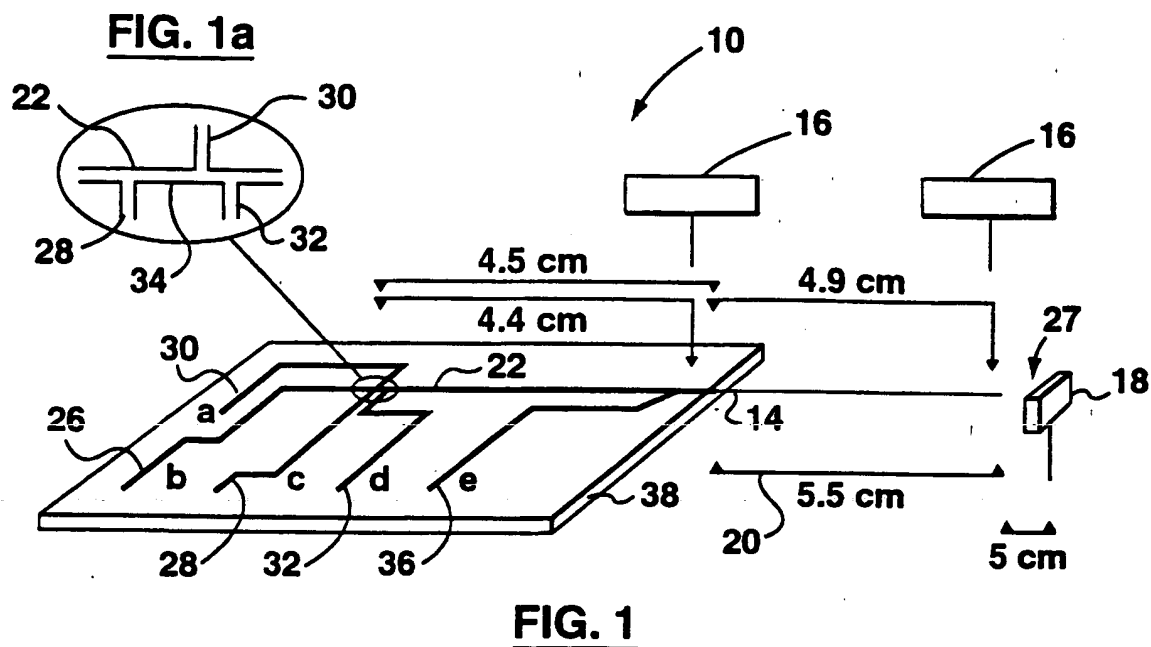
(2) Providing a side channel connecting to the main
channel adjacent the capillary tube; and

(3) Passing a buffer solution through the side channel, to
increase the flow rate from the tip of the capillary tube.

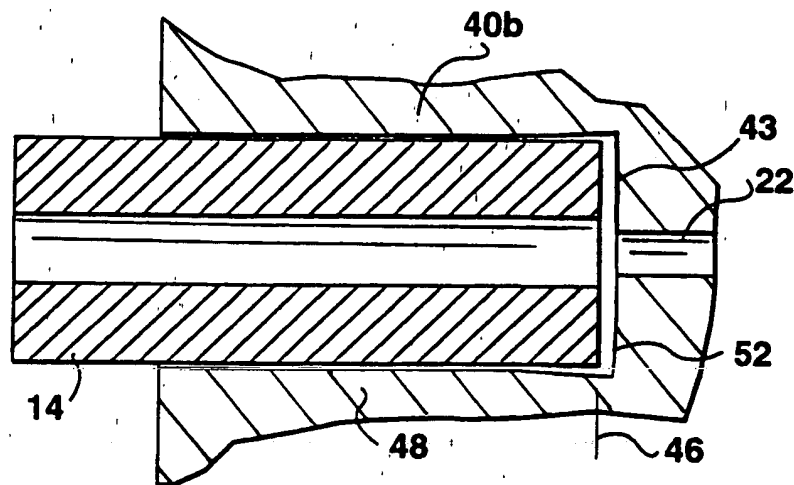
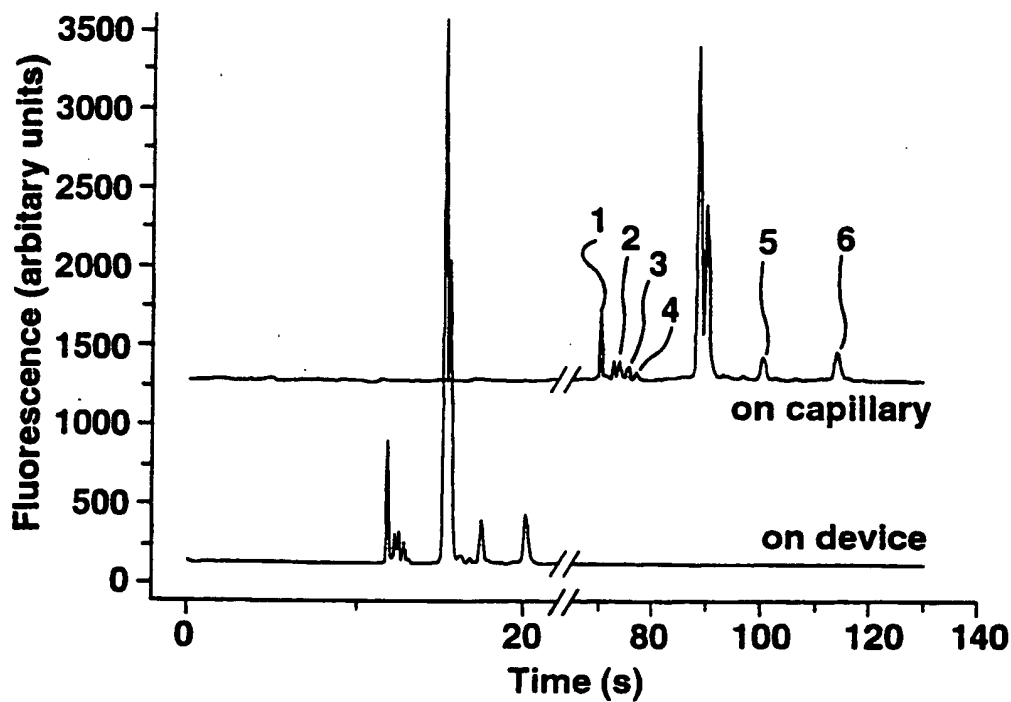
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**FIG. 2**

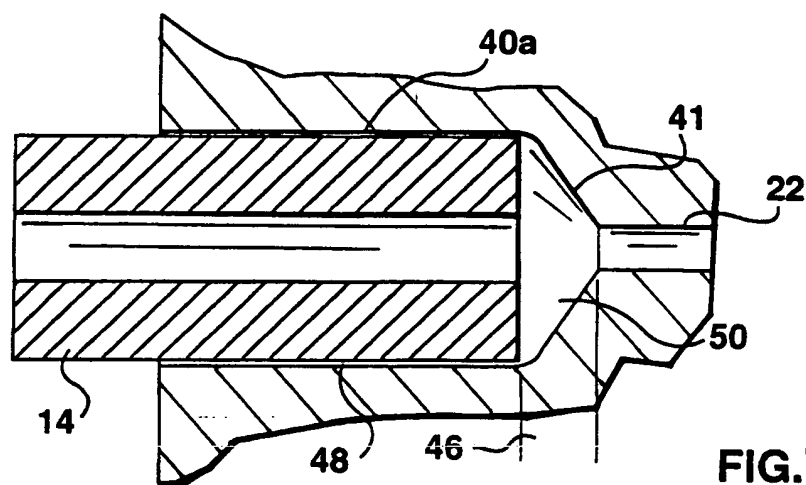
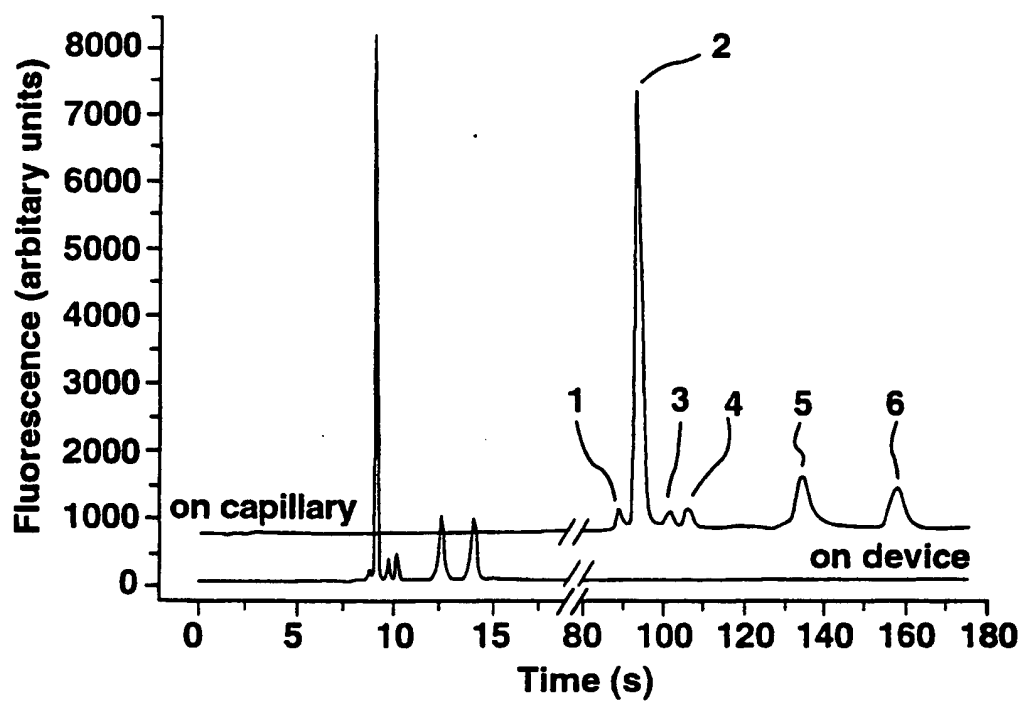
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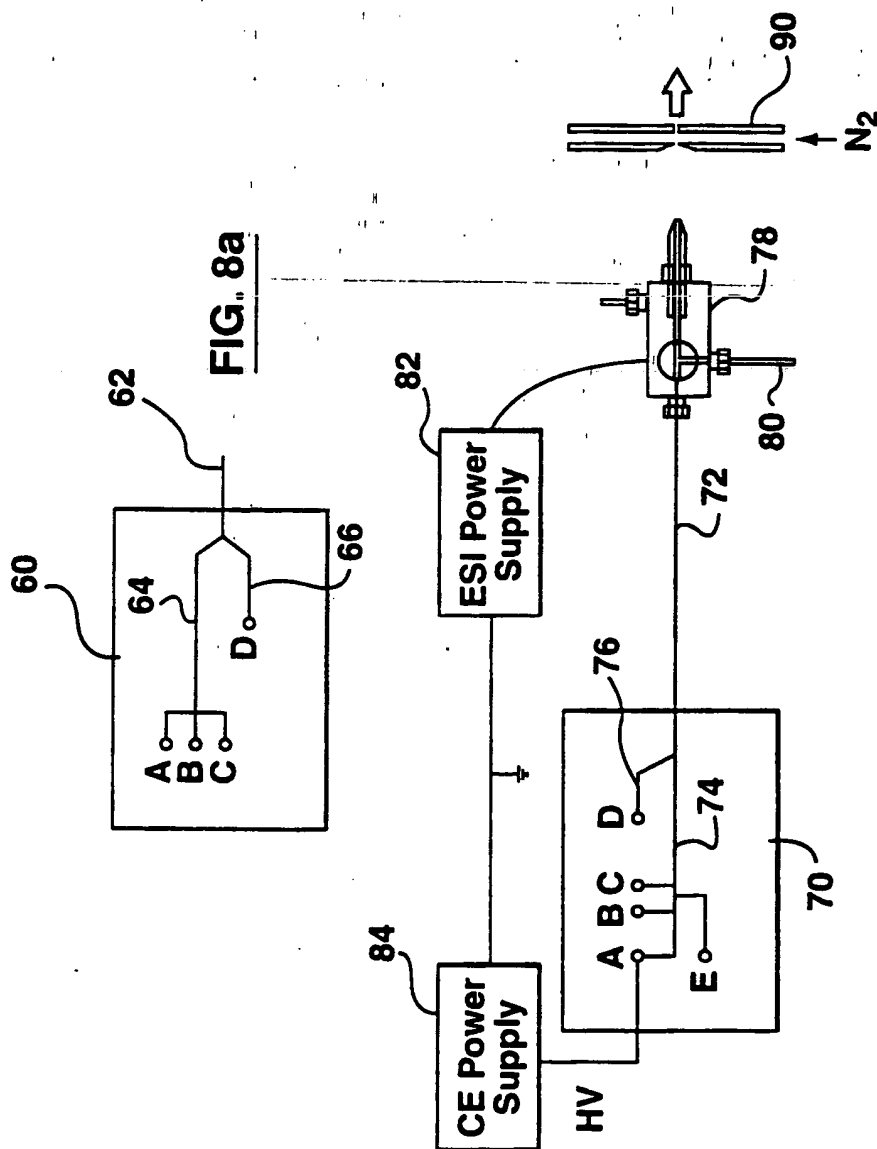
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**FIG. 4****FIG. 5**

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**FIG. 6****FIG. 7**

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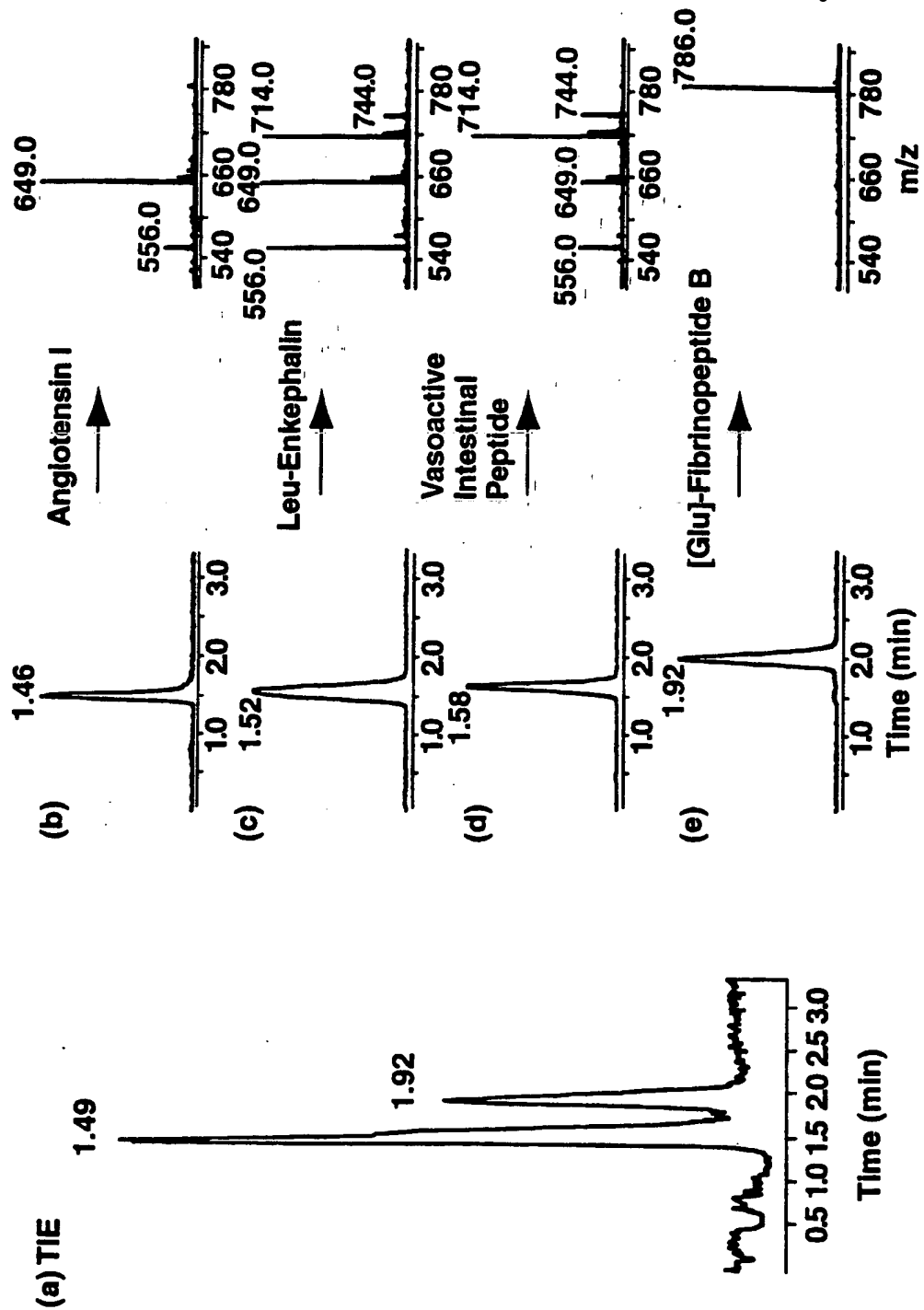


FIG. 9

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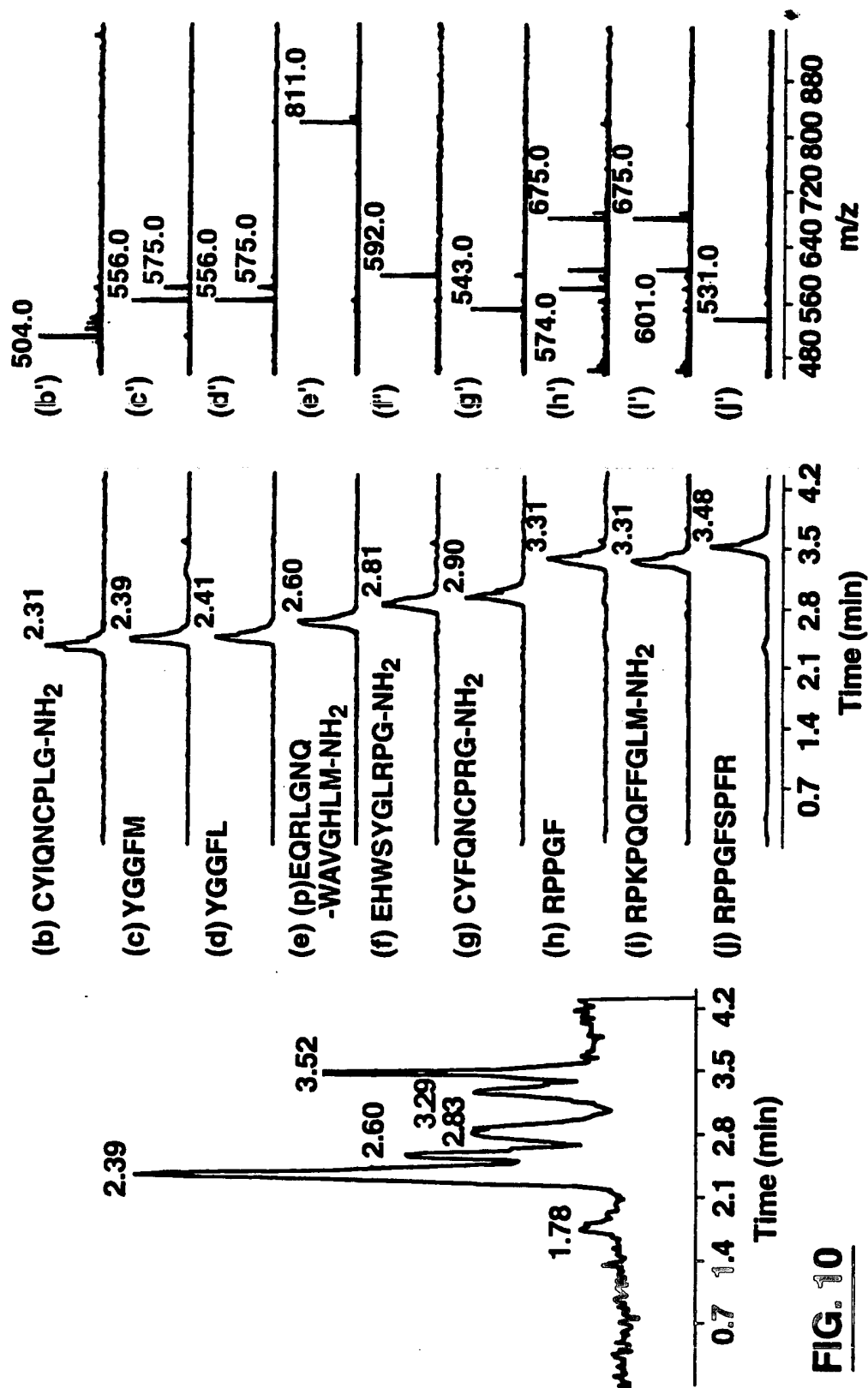
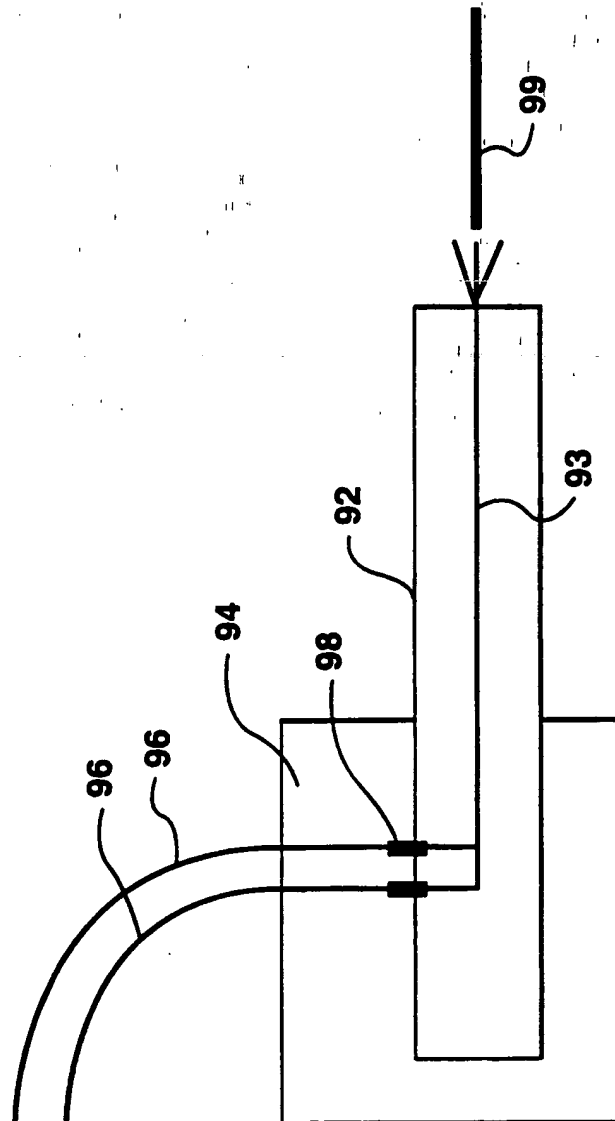


FIG. 10

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**FIG. 11**



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<p>(21) International Application Number: PCT/CA99/00868 (22) International Filing Date: 23 September 1999 (23.09.99) (30) Priority Data: 09/169,146 9 October 1998 (09.10.98) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 09/169,146 (CON) Filed on 9 October 1998 (09.10.98) (71) Applicants (for all designated States except US): UNIVERSITY OF ALBERTA [CA/CA]; Faculty of Science, Department of Chemistry, E3-44 Chemistry Building, Edmonton, Alberta T6G 2G2 (CA). INSTITUTE FOR BIOLOGICAL SCIENCES [CA/CA]; National Research Council, 100 Sussex Drive, Ottawa, Ontario K1A 0R6 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): TANG, Thompson [CA/CA]; #306-10713 - 80 Avenue, Edmonton, Alberta T6E 1V7 (CA). HARRISON, D., Jed [CA/CA]; W3-13 Chemistry, University of Alberta, Edmonton, Alberta T6E 1V7 (CA). BINGS, Nicolas [CA/US]; c/o Prof. Gary</p>	<p>Hieftje, Department of Chemistry, Bloomington, IN 47405 (US). WANG, Can [CA/CA]; 3A, 9111-112 Street, Hub Mall, Edmonton, Alberta T6G 2C5 (CA). OCVIRK, Gregor [CA/CA]; 10539 - 85 Avenue #9, Edmonton, Alberta T6E 2K5 (CA). LI, Jianjun [CN/CA]; 101 Boul. Sacre Coeur, Hull, Quebec J8X 1C7 (CA). SKINNER, Cameron [CA/CA]; #204 Vanier Hse., Michener Pk., Edmonton, Alberta T6H 4N1 (CA). THIBAUT, Pierre [CA/CA]; 218 des Explorateurs, Aylmer, Quebec J9J 1M9 (CA). (74) Agent: BERESKIN & PARR; 40th floor, 40 King Street West, Toronto, Ontario M5H 3Y2 (CA). (81) Designated States: CA, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report. (88) Date of publication of the international search report: 13 July 2000 (13.07.00)</p>	
<p>(54) Title: MICROFLUIDIC DEVICES CONNECTED TO CAPILLARIES WITH MINIMAL DEAD VOLUME</p>		
<p>(57) Abstract</p> <p>A method is provided for joining a microchip device to a capillary tube. The microchip device has a capillary channel opening onto an edge surface of the device. A short hole is drilled into the edge surface, aligned with the capillary channel. The drilling is done with a flat bottom, preferably by a two-step drilling process. Then, the end of the capillary can be inserted into the hole so that its end is substantially flush with the flat bottom of the hole, thereby eliminating dead volume. Testing has shown that this connection provides very little band broadening of samples transported through the capillary channel into the capillary tube. The tip of the capillary tube can be tapered, so that it is suitable for use as an electrospray source for a mass spectrometer.</p>		

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 99/00868

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 H01J49/04 G01N30/60 G01N27/447 B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

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